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A VALIDATED SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF DUTASTERIDE IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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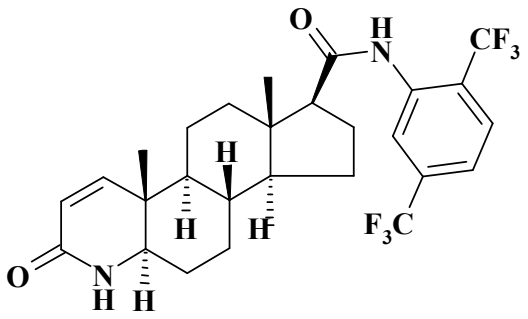
ABSTRACT: A simple, sensitive and accurate UV spectrophotometric method has been developed for the determination of dutasteride in raw material and tablets. The drug showed maximum absorption at 240 nm. The method was found linear in the range 5-50 µg mL^{-1} for the drug with Sandell sensitivity of 0.0522 µg cm^{-2}/0.001A. The limits of detection and quantification were found to be 1.23 and 3.75 µg mL^{-1}, respectively. Results were validated statistically according to ICH guidelines. Validation of the method yielded good results concerning range, linearity, precision and accuracy. It was found that the excipients present in the commercial formulation did not interfere with the method.
KEYWORDS: Dutasteride, UV spectroscopy, ICH guideline, Validation.

INTRODUCTION

Dutasteride is a potent and specific dual 5α-reductase inhibitor for the treatment of benign prostatic hyperplasia (BPH) and lower urinary tract symptoms (LUTS)[1, 2]. It was approved by USFDA in October 2002 and it has been approved in several countries[3, 4]. Dutasteride inhibits the conversion of testosterone to 5α-dihydrotestosterone (DHT).[2] DHT is the androgen, which is primarily responsible for the initial development and subsequent enlargement of the prostate gland.

Figure 1: Chemical structure of Dutasteride



Chemically dutasteride is 17β-N-(2,5-bis (trifluoromethyl) phenyl-carbamoyl)-4-aza-5-androst-1-en-3-one[5] (Figure 1). The drug is not official in any pharmacopoeia till date. A survey of literature has not revealed any UV spectrophotometric method for the determination of the drug in bulk or pharmaceutical formulation. The other analytical methods that have been reported for its determination in bulk and pharmaceutical formulation include HPLC[6] and HPTLC[7]. A liquid chromatography–tandem mass spectrometry (LC-MS/MS) method using sophisticated LC–MS/MS apparatus has also been reported for quantification of the drug from human plasma[8]. So there is a need to develop a spectrophotometric method for the determination of the same.

In the present study, a simple, economical, precise and accurate analytical method for the estimation of dutasteride in pure form and in solid dosage form was developed. The results of the analysis were validated by latest guidelines set by International Conference on Harmonization (ICH)[9].

MATERIALS AND METHODS

Dutasteride reference substance was obtained from Intas Pharmaceuticals Ltd., (Ahmedabad, India). Tablets of brand Veltride (Batch No G0106, Intas

Pharmaceuticals Ltd., Ahmedabad, India) containing 0.5 mg of dutasteride were procured from a local pharmacy. The solvent used for the experiment was methanol (AR grade, Merck, India).

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Precisa 310M, Switzerland).

Dutasteride stock solution (100 µg mL^{-1})

The standard solution of dutasteride was prepared by accurately weighing 10 mg of the drug and was diluted in 100 mL volumetric flask with methanol to produce a stock solution of 100 µg mL^{-1}. This stock solution was used to prepare further dilutions of standard solution.

Estimation of optical wavelength (λ_{max}) of maximum absorption of dutasteride

Aliquots of stock solution of dutasteride were transferred into a series of 25 ml volumetric flasks and volume was made up to the mark methanol to produce the concentration ranging from 5-50 µg mL^{-1}. From the UV absorption spectra 240 nm was selected as λmax (Fig. 2) obtained by scanning the pure drug solutions in the range of 200-400 nm for measuring the absorbance of above solutions to prepare the calibration curve.

Calibration curve

Calibration curve was plotted in the concentration range of 5-50 µg mL^{-1} by diluting the standard stock solution with phosphate buffer. The absorbance was measured at 240 nm against the corresponding solvent blank. The linearity of the plot between absorbance and the concentration of the drug in the concentration range 5-50 µg mL^{-1} was calculated to obtain the calibration curve as well as the regression coefficient (r^2).

Estimation of dutasteride from commercial preparation

For the analysis of the dosage form, forty tablets of dutasteride (0.5 mg) were ground to fine powder and mixed thoroughly. Powder equivalent to 10 mg of the drug was transferred to a 100 mL volumetric flask and dissolved in about 40 mL methanol by shaking on a thermostatically controlled water bath for 30 min. The solution was filtered through Whatman filter paper (No. 41). The filter paper was washed with methanol. The washings were added to the filtrate and the final volume was made up to 100 mL with the blank. After suitable dilution, the absorbance of final sample was recorded against the blank at 240 nm. All determinations were conducted in triplicate (Table 2).

Validation of method by ICH guideline

The method was validated according to the guidelines set on the International Conference on Harmonization (ICH)[9] for the validation of the analytical procedures. The parameters, which were used to validate the method of analysis, were linearity, range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), specificity and robustness.

The linearity response was assessed in the range of 5-50 µg mL^{-1}. Appropriate amounts of the stock solution were diluted with methanol, yields concentrations of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µg mL^{-1}. Absorbance of the standard solutions was plotted against theoretical concentration. The linearity was evaluated by calculation of the correlation coefficient obtained from linear regression analysis.

The repeatability of the analytical method was assessed by assaying six sample solutions of dutasteride (20 µg mL^{-1}) during the same day under same experimental conditions. Intermediate precision was evaluated by analyzing the solutions on three different days. Absorbance was determined and compared. Precision was expressed as percentage relative standard deviation (RSD).

The accuracy of the developed method was evaluated through recovery test by standard addition method. Sample solution of dutasteride having concentration (20 µg mL^{-1}) was spiked with three known concentrations of reference standard at three different levels lower, medium, and upper concentration. The recovery of the added reference standard was determined in triplicate and calculated by the following formula:

% Recovery = $100 \times (S_p - S_t)/S_s$ in which S_p is the concentrations of spiked solution, S_t is the reference standard solution and S_s is the sample solution

RESULTS AND DISCUSSION

The proposed method is simple, rapid and precise and does not suffer from any interference due to the common excipients of tablet. The drug was analyzed by the proposed method both as a raw material and as a pharmaceutical tablet formulation. The linear regression equation was calculated to be Y= 0.0184X+ 0.0125 where X and Y are concentration in µg mL^{-1} and absorbance respectively.

A standard calibration curve of the drug was constructed by plotting absorbance versus concentration (Figure 3). The UV absorption spectrum (Figure 2) was monitored at 240 nm. Different optical characteristics of the proposed method are summarized in table 1. Agreement with Beer's law was evident from the concentration range of the final dilution of 5-50 µg mL^{-1} with an apparent molar absorptivity and Sandell sensitivity of 1.0122×10^4 $L.mol^{-1}cm^{-1}$ and 0.0522 µg cm^{-2}/0.001A, respectively. The limits of detection (LOD) and quantification (LOQ) are

calculated to be 1.23 and 3.751µg mL^{-1}, respectively. The correlation coefficient obtained for the line was 0.9995 indicating very good linearity. The method had excellent reproducibility for standard solution of 20 µg mL^{-1}. The average purity was reached 98.342%.

In this test the observed concentrations of dutasteride reference substance in the tablets were not significantly different from the stated concentrations by Student's t test, p = 0.05 (100.317 ± 1.011 %). The percentage recovery value (table 3), which is close to 100 %, indicates the accuracy of the method and absence of interference of the excipients present in the formulation.

The precision was expressed as the percent coefficient of variation of each curve. The ANOVA analysis (Table 4) showed there is no significant difference (F_{stat} value < tabulated F value at p = 0.05) among the assay results obtained in three different days at different times. No interfering intensity was found in the UV spectra due to the tablet excipients. Dutasteride was shown to be stable during all procedure.

CONCLUSION

The proposed method is simple, selective, and rapid and can be used for routine quality control measurements. This method is found to be applicable for the analysis of dutasteride in bulk drugs and pharmaceutical products like tablets. The method is free from interferences present in the sample. Moreover the present technique has the advantage of using inexpensive and easily available reagent; does not require formation of any colored complex that causes variation in the intensity of the color with time or sophisticated instruments like HPLC or HPTLC; therefore can be frequently used in the laboratories of research, hospitals and pharmaceutical industries.

Table 1: Optical characteristics of the Proposed method

Parameters	Data
Absorption maxima (nm)	240 nm
Beer's law limit (µg /ml)	5-50
Correlation coefficient (r)	0.9995
Molar absorptivity ($l.mol^{-1}cm^{-1}$)	1.0122×10^4
Regression equation (Y=mX + C)	Y= 0.0184X+ 0.0125
Sandell sensitivity (µg cm^{-2}/0.001A)	0.0522
Slope (m)	0.0184
Intercept (c)	+ 0.0125
Detection limit (LOD) (µg /ml)	1.23
Limit of quantification (LOQ) (µg /ml)	3.751
% Recovery ± S.D.	100.091 ± 0.78

Table 2: Analysis of dutasteride tablets (0.5 mg)

Sl. No	A*	%Analysis ± S.D.	SEM	% C.V.
1	0.377	100.317±1.011	0.452	1.008
2	0.378			
3	0.382			
4	0.382			
5	0.384			
6	0.387			

*Absorbance – Average of three determinations, S.D.: Standard deviation, SEM: Standard Error of Mean, C.V.: Coefficient of Variance.

Table 3: Recovery studies of dutasteride tablets

Sl. No.	Spiked amount (µg mL^{-1})	Recovery amount (µg)	Recovery (%)	Recovery (%) ± S.D.
1	25	25.1359	100.5435	100.091 ± 0.78
2	30	29.7554	99.1848	
3	35	35.1902	100.5435	

Table 4: ANOVA of intra- and inter-day assay of dutasteride tablets

Source of Variation	SS	df	MS	F_{stat}*	F at level 1%	F at level 5%
Inter-day assay	1.1076	4	0.2769	5.7692	0.0590	6.3882
Intra-day assay	0.0295	1	0.0295	0.6153	0.4766	7.7086
Residual	0.1919	4	0.0479			
Total	1.3291	9				

*F_{stat} < F at level 1% and 5% in both cases.

Figure 2. UV spectrum of Dutasteride measured in methanol.

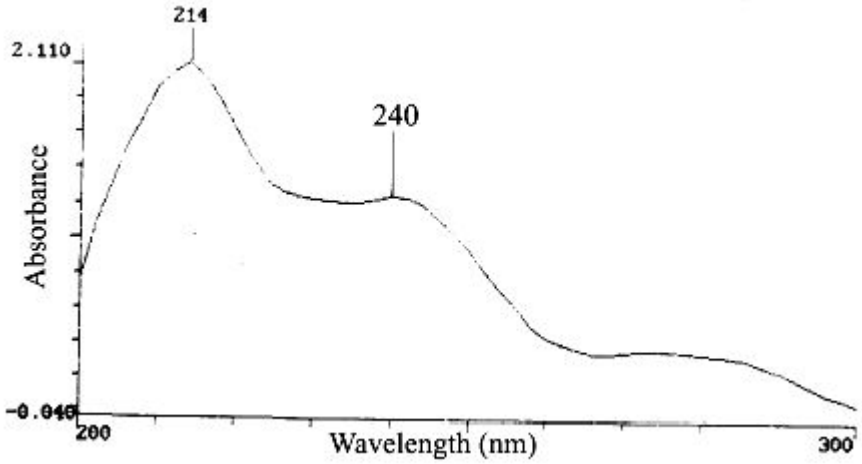
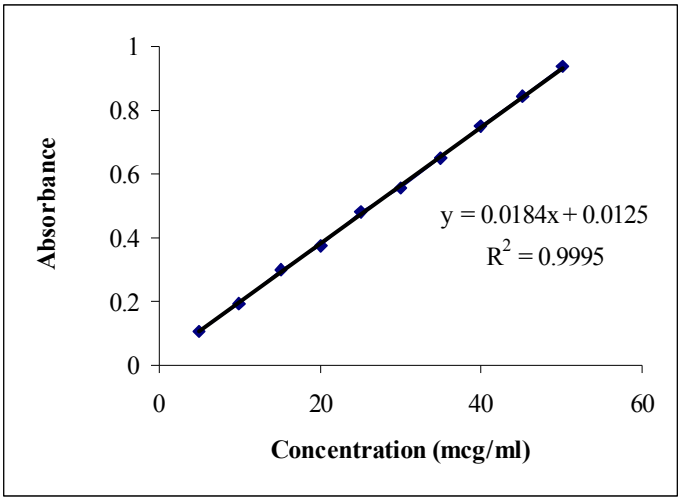


Figure 3: Calibration curve of Dutasteride against methanol as blank.



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